2542-Pos Board B512

Structural Dynamics Of The Actin Binding Cleft Of *Dictyostelium* Myosin II Analyzed By Stopped Flow Time-Resolved FRET

Joseph Muretta, Jennifer C. Klein, David Kast, Bengt Svensson, David D. Thomas.
University of Minnesota, Minneapolis, MN, USA.

Using site-directed fluorescence labeling, transient time resolved FRET ($[TR]^2$FRET) and time resolved fluorescence anisotropy, we have measured actin- and nucleotide-induced structural changes within the actin binding cleft of *Dictyostelium* myosin II. In a recent report using time-resolved pulsed EPR spectroscopy, distances were measured between paramagnetic probes attached to the upper and lower 50 kDa subdomains of *Dictyostelium* myosin II (Klein et al., *PNAS*, 105:12867-72). These results support the hypothesis that the actin-binding cleft closes partially upon actin binding, but also suggested that both open and closed conformations are simultaneously present, with nucleotides and actin controlling the open-closed equilibrium. Due to technical constraints, those EPR distance measurements were limited to frozen samples. In the present study, time-resolved fluorescence is used to probe the cleft in solution under more physiologically relevant conditions, including the transient phase of the ATPase reaction. Fluorescent probes were attached to engineered Cys residues in the upper and lower 50 kDa subdomains and used to measure the distance across the cleft. Single probes attached to either subdomain were used in combination with fluorescent nucleotides to monitor the coupling between the actin-binding cleft and the active site. Here we use a combination of transient time resolved FRET and transient time resolved fluorescence anisotropy to probe the equilibrium between open and closed cleft conformations. In the key experiments, a complete nanosecond time-resolved fluorescence decay was measured, defining the detailed distance distribution between probes, every 0.1 ms following rapid mixing (stopped flow), thus yielding high-resolution structural information on the sub-millisecond time scale. The results provide new insights into the coupling between the actin-binding cleft, the active site, and actin binding.

2543-Pos Board B513

Structural Dynamics of the Myosin Relay Helix Resolved by DEER and Time-Resolved FRET

Roman Agafonov, Yuri E. Nesmelov, Sarah Blakely, Margaret A. Titus, David D. Thomas.
University of Minnesota, Minneapolis, MN, USA.

We have used DEER (double electron-electron resonance) and TR-FRET (time-resolved fluorescence resonance energy transfer) to study conformational changes within the myosin II relay helix. Major structural changes during the myosin II ATPase cycle take place in the force-generating domain. Crystal structures show that the converter domain, the relay helix, and SH1 helix have different conformations in the proposed pre- and post- powerstroke structural states of myosin. This dramatic structural change is detected in solution studies by intrinsic fluorescence [1, 2] and EPR of a spin label attached to SH1 [3]. In the present study, we focus on the relay helix as a crucial structural element involved in coupling between the force-generating domain and the nucleotide-binding pocket. Cysteine mutations were introduced into a Cys-lite construct of *Dictyostelium discoideum* (*Dicty*) myosin in the lower 50k domain (either D515C or A639C) and the C-terminal end of the relay helix (K498C). These constructs were selectively modified with either MSL/MSL or IAEDANS/DABCYL pairs, and the distance between probes was measured in different myosin conformations trapped with nucleotides or nucleotide analogs Two conformations of the relay helix (with distinct probe-to-probe distances, presumably corresponding to the ''straight'' and ''bent'' states of the relay helix) were resolved. Observed distances were in good agreement with existing crystal structures, but at least two distinct structural states were present in certain biochemical states (e.g, with bound ADP.BeF_3, ADP.V_i, ADP.AlF_4). The mole fraction of the ''bent'' conformation was higher with post-hydrolysis analogs (ADP.V_i, ADP.AlF_4) bound at the active site. Our results reveal structural rearrangements within a single subdomain of myosin and provide insights into the coupling between ATP binding and changes in the force-generating region.

2544-Pos Board B514

A Glimpse at Loop 1 Movement in Smooth Muscle Using Intrinsic Tryptophan Fluorescence

Justin Decarreau, Lynn Chrin, Chris Berger.
University of Vermont, Burlington, VT, USA.

Smooth muscle myosin has two N-terminal isoforms that result from alternative splicing of loop 1. Loop 1 contains a seven amino acid insert (QGPSFSY) in one isoform (SM-B) that is absent in the other (SM-A). It has been shown that the presence of the insert causes a two-fold increase in the rate of in-vitro actin sliding velocity and actin-activated ATPase (Rovner *et al.*, *J. Muscle Res. Cell Motil.* **18**:103, 1998). Based on these results and its proximity to the active site it was hypothesized that loop 1 plays a role in modulating the release of ADP (Spudich, *Science* **372**:515, 1994). However, little is known about the conformation of loop 1 in different nucleotide states, as it is absent in crystal structures. To examine the position of loop 1 and its potential role in ADP release we have engineered a single tryptophan residue into loop 1 at position 215. Using the intrinsic tryptophan fluorescence from W215 and fluorescent analogs of ADP and ATP we have looked at the position of loop 1 as a function of temperature. The results suggest two conformations of loop 1 in the ADP state, both an open and closed form. The distance between loop 1 and the active site decreases for both nucleotides from 25-15°C. At 10 °C loop 1 moves away from the active site in the ADP state, while there is no additional movement in the presence of ATP. This is the first data to demonstrate movements of loop 1 associated with different nucleotide states of the myosin active site, giving insight into how it may contribute to nucleotide release.

2545-Pos Board B515

Importance In The Powerstroke Of Interaction Between The Relay Helix And Helix HQ Of Myosin

Conor Doss[1], Lisa Goddard[1], Annica Stull-Lane[1], Kathryn Chenault[1], Katherine Erickson[1], Don Moerman[2], **Taylor Allen**[1].
[1]Oberlin College, Oberlin, OH, USA, [2]University of British Columbia, Vancouver, BC, Canada.

Ideas on mechano-chemical transduction by myosin have matured greatly through crystallography and are ripe for testing *in vivo*. One approach for doing so is termed reversion analysis, in which pairs of compensating mutations are identified. Suppression of one missense mutation by another reveals an interaction at the amino acid level that may be direct or indirect, long-lived or fleeting, but nonetheless physiologically relevant. Reversion analysis thus can test current ideas and, importantly, uncover interactions underlying dynamic or strain-dependent myosin states likely not represented in crystals. We used random mutagenesis to induce suppressors of *Caenorhabditis elegans* myosin/UNC-54 mutation E524K (=E500 of chicken myosin V), located on helix HQ at the predicted actin-binding region.

Worms with E524K alone display disorganized A-bands and have a paralysis that worsens with increasing temperature. Thermodynamically, the heat-sensitivity suggests loss of a salt-bridge. The comparable residue in other myosins forms in the rigor-like and post-rigor crystallographic structures, but not in the pre-powerstroke one, a salt-bridge with a lysine on the relay helix (K460 of myosin V; =K483 of UNC-54). Thus, in the paralyzed worms, electrostatic repulsion between E524K and K483 potentially destabilizes interactions between helix HQ and the relay helix, thereby hindering the powerstroke.

Twenty independent lines of suppressed worms were recovered from a screen of 10^6 mutagenized haploid genomes, and the suppressors mapped to seven residues: near the P-loop, V187I; in the actin-binding domain, E524K to E/T, L547F, A548V, and M579I/L/V; on the SH1-helix, C712Y; and in the converter domain, D724N. Consistent with the crystallographic structures of myosin, the suppressors can be interpreted as diminishing unfavorable interaction between E524K and K483, thus permitting the relay helix to reorganize properly as myosin progresses through the crossbridge cycle.

2546-Pos Board B516

New Mechanism of Actin Activation of Myosin

Boglarka Varkuti, Balint Kintses, Laszlo Vegner, Anna Rauscher, **Malnasi-Csizmadia Andras**.
Eotvos University, Budapest, Hungary.

Actin activation of myosin ATPase activity is a general property of actomyosin systems, however, its role in the mechanochemical transduction is still not unveiled. Recently, we showed that in the absence of actin the rate limiting step of the ATPase cycle is not the phosphate release step but the preceding conformational change and actin activates directly this step to constitute the power stroke. Here we report that the rate of the power stroke step is initiated directly by the interaction of actin and the prolin rich (PR) loop which is located in the proximal part of the relay helix. Deletion of the PR loop does not change any of the kinetic properties of *Dictyostelium* myosin motor domain in the absence of actin. Actin binding of the PR loop deleted mutant decreased only three fold in rigor and in the presence of nucleotides. Also, ATP induced actin dissociation was only slightly affected by the deletion. Nevertheless, we found that PR loop deletion caused dramatic effect on the actin activation: basal ATPase activity is not increased by actin. Surprisingly, the motility is not reduced if this interaction between the PR loop and the